DECLARATION

I, Mayumi Takano, of Yanagida & Associates, 7F Shin-Yokohama KS Bldg., 3-18-3 Shin-Yokohama, Kohoku-ku, Yokohama-shi, Japan, hereby certify that I understand both English and Japanese, that the translation is true and correct, and that all statements are being made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Mayumi Takano

Mayumi Takano

Dated this 30th day of January, 2006

[Name of Document] SPECIFICATION

[Title of Invention] Test piece and analysis method using the test piece

[Scope of Demand for Patent]

1. A test piece comprising

a plurality of probes arranged and immobilized thereon, to some of which a target substance marked with a marker binds, and

a pattern of ID information peculiar to the test piece attached to a predetermined location on the test piece.

2. A test piece according to Claim 1, wherein the ID information is comprised of a marker the same as or similar to the marker used for marking the target substance.

3. A test piece according to either Claim 1 or 2, wherein the pattern of the ID information is attached on the test piece using a spotting device or an ink jet printer.

4. An analysis method for analyzing a target substance comprising the steps of

causing the target substance marked with a marker to bond to some of a plurality of probes arranged and immobilized on a test piece by bringing the target substance marked with the marker into contact with the test piece,

obtaining information concerning positions of the probes to which the target substance has bonded,

attaching ID information peculiar to the test piece at a predetermined location on the test piece before obtaining the information concerning the positions of the probes, and

reading out the ID information.

5. An analysis method according to Claim 4, wherein the ID information is comprised of a marker the same as or similar to the marker used for marking the target substance.

[Detailed Description of the Invention]

[0001]

[Technical Field of the Invention]

The present invention relates to an analysis method using a test piece such as a micro-array, a macro-array or a DNA chip. More specifically, the present invention relates to: a test piece having a base carrying plural types of probes (e.g., organic molecules) arranged and immobilized thereon and identifying positions of those probes on the test piece to which a target substance which includes organic molecules marked with a marker (e.g., radioactive isotope or fluorescent dye) has hybridized; and an analysis method using the test piece.

[0002]

[Description of the Related Art]

A method for analyzing gene expressions using a micro-array is described in detail in the paper titled "Gene

Expression Analysis Using the Micro-Array" (Experimental Medical Science Series Vol. 17; p.61-65; Yodosha Shuppann, Inc. (January, 1999).

[0003]

Such methods of gene expression analysis using the micro-array, a macro-array, a DNA chip, etc., have recently come into wide use. A test piece as shown in Figure 8 is used in those methods. The test piece has a base plate 40 (e.g., a membrane, a glass plate, a glass slide or a silicon base plate) on a surface of which plural kinds of organic molecules (frequently used are, e.g., c-DNA, oligo-DNA, other types of DNA, PNA and EST) are arranged and immobilized in matrix as probes using a spotting device etc. Such a test piece may be referred to by either of a variety of names (e.g., a macro-array, a micro-array or a DNA chip) according to the type or size of the base plate 40, the number of array-points, size of each array-point, the types of the substances used as the probes, the type of the target substance, etc.

[0004]

On the other hand, the target substance contains organic molecules (e.g., molecules of c-DNA, genom-DNA, m-RNA, other types of RNA, dNTP or PNA) marked with radioactive isotope, fluorescent dye, etc.

[0005]

In the analysis, the target substance marked with markers such as the radioactive isotope hybridizes with the probes immobilized in a matrix.

[0006]

In case the organic molecules capable of hybridizing (or combining) with each other are included in the probes and the target substance, both organic molecules hybridize (or combine) with each other on the base, and thereby the marker such as the radioactive isotope or the fluorescent dye is immobilized to the several probes containing the hybridized organic molecules on the base through the organic molecules. On the other hand, the marker such as the radioactive isotope or the fluorescent dye would not be immobilized to those probes which have not hybridized with organic molecules. Double circles in Figure 8 schematically indicate the positions on the base where the hybridized probes reside, i.e., the positions where the marker such as the radioactive isotope or the fluorescent dye is immobilized. Although each of the array-points arranged in matrix is clearly separated from others for explanatory purposes in Figure 8, each of the array-points can hardly be distinguished visually from others in practice as they are very small and arranged in close proximity to each other at a high density.

[0007]

The positions of the hybridized probes on the base can be identified by detecting the positions on the test piece where the marker such as the radioactive isotope or the fluorescent dye is immobilized. Subsequently, the types of the hybridized probes can be identified based on the identified positions thereof. At the same time, the amount of the hybridized target substance can be detected.

[0008]

[Problems to be solved by the Invention]

As described above, the process of identifying the types of the hybridized probes always includes the step of comparing two sets of information, i.e., one set of information concerning the detected positions of the marker such as the radioactive isotope or the fluorescent dye and the other set of information concerning the type and the position of each probe arranged and immobilized on the test piece.

[0009]

However, it has been typical that the set of information concerning the type and the position of each probe arranged and immobilized on the test piece is kept stored in the spotting device etc. used for preparing the test piece. For this reason, an operator has heretofore been required to manually input to a computer the set of information concerning the type and the position of each probe arranged and immobilized on the test

piece to identify the types of the hybridized probes based on the set of information concerning the detected positions of the marker such as the radioactive isotope or the fluorescent dye. Hence, when conducting experiments on a plurality of test pieces each carrying several types of probes arranged and immobilized thereon in a different pattern, it has sometimes occurred that the operator inputs the set of information concerning the wrong test piece or that the experiment is carried out on the wrong test piece. In those cases, correct combinations cannot be obtained between the information concerning the type and the position of each probe arranged and immobilized on the test piece and the information concerning the positions of the detected marker.

[0010]

The object of the present invention is to provide: a test piece effective in preventing incorrect association between two sets of information, i.e., one set of information concerning the types and the positions of a plurality of probes arranged and immobilized on the test piece and the other set of information concerning positions of the detected marker; and an analysis method using the test piece.

[0011]

The field of use of the test piece according to the present invention is not necessarily limited to the field of gene

analysis, e.g., in the field of gene expressions analysis, base sequence identification, variation analysis or polymorphism analysis. The test piece of the present invention can instead be used for analysis of any target substance as long as the target substance is capable of selectively bonding to the probes, which are arranged and immobilized in matrix on a base, through any kind of reaction.

[0012]

[Means used to solve the Problems]

A test piece according to the present invention comprises: a plurality of probes arranged and immobilized thereon, to some of which a target substance marked with a marker binds; and a pattern of ID information peculiar to the test piece attached to a predetermined location on the test piece.

[0013]

The ID information is preferably comprised of a marker the same as or similar to the one used for marking the target substance.

[0014]

The test piece according to the present invention may take any form, e.g., the form of a micro-array, a macro-array or a DNA chip, as long as it remains capable of carrying a plurality of probes arranged thereon.

[0015]

The substances used as the probes may be of any kind, e.g., c-DNA, oligo-DNA, other types of DNA, PNA or EST, as long as it is suitable for use in array analysis. That is to say, the substance arranged and immobilized as a probe on the test piece of the present invention is not limited to organic molecules but may be of any kind as long as it remains capable of being combined selectively with the target substance through any kind of reaction.

[0016]

Typical examples of the target substance include organic molecules such as the molecules of c-DNA, genome-DNA, RNA such as m-RNA, dNTP or PNA. However, any appropriate substance other than those organic molecules listed above may also be used.

[0017]

The "predetermined location" may be any location on the test piece other than those spots where the probes are arranged and immobilized.

[0018]

The term "ID information" refers to information for distinguishing each test piece from others. The information is necessary at least for identifying the type and the position of each probe arranged and immobilized on that test piece, so the ID information may be an ID number, etc. attached to each test piece, for example. The ID information may include

information, such as the type of the base of the test piece, the date of preparing the test piece, a serial number, a lot number, the positions of the probes and the like. The ID information may additionally include information concerning the target substance as well. Herein, it is preferable to have the ID information as an encoded form in order to minimize the space on the test piece required for attaching the ID information when the ID information is arranged on the predetermined position of the test piece.

[0019]

Typical examples of the marker include radioactive isotopes and fluorescent dyes. However, any other appropriate marker may also be selected.

[0020]

The pattern of the ID information peculiar to the test piece may be arranged and fixed on the test piece using a spotting device, an ink jet printer, etc.

[0021]

An analysis method for analyzing a target substance according to the present invention comprises the steps of: causing the target substance marked with a marker to bond to a plurality of probes arranged and immobilized on a test piece by bringing the target substance marked with the marker into contact with the test piece; obtaining information concerning

positions of the probes to which the target substance has bonded; attaching ID information peculiar to the test piece at a predetermined location on the test piece before obtaining the information concerning the positions of the probes and reading out the ID information.

[0022]

The possible forms of bonding between the target substance and the probes include hybridization, in which a pair of complementary base sequences forms a stable double strand, and any other specific form of bonding. The possible types of reactions through which the target substance bonds to the probes include, for example, several types of affinity.

[0023]

The information concerning the positions of the probes to which the target substance has bonded refers to information indicating the position of the hybridized probes, which can be obtained by detecting the positions of the marker immobilized to the probes hybridized with the target substance.

[0024]

In the method for analyzing the target substance according to the present invention, the ID information is preferably comprised of a marker the same as or similar to the one used for marking the target substance.

[0025]

In this context, "a marker similar to the one used for marking the target substance" refers to a marker which is different from the marker used for marking the target substance but which emits radiation similar to the radiation emitted by the marker used for marking the target substance, in the case where a radioactive isotope is selected as the marker used for marking the target substance, or refers to a marker which is different from the marker used for marking the target substance but which emits fluorescence having a wavelength range similar to the fluorescence emitted by the marker used for marking the target substance when exposed to stimulating light having a wavelength range similar to that for the marker used for marking the target substance, in the case where a fluorescent dye is selected as the marker used for marking the target substance.

[0026]

The step of attaching the ID information to the predetermined position on the test piece may be carried out at any point in time before the step of obtaining the information concerning the positions of the probes to which the target substance has bonded. For example, the ID information may be attached upon arrangement of the probes on the test piece or may be attached after the step of causing the target substance to bond to the probes.

[0027]

[Advantageous Effects of the Invention]

According to the analysis method of the present invention in which the ID information peculiar to the test piece is arranged on the test piece, incorrect association between one set of information concerning the detected positions of the probes to which the target substance has bonded and the other set of information concerning the type and the position of each probe arranged and immobilized on the test piece may be prevented effectively, by detecting the information peculiar to the test piece at the time of detecting the marker on the test piece and accordingly, correlating the information concerning the positions of the probes to which the target substance has bonded with the ID information.

[0028]

In this respect, the ID information and the information concerning the positions of the probes to which the target substance has bonded may be detected simultaneously requiring no additional step, i.e., the step of reading out the ID information may be incorporated into the step of obtaining the information concerning the positions of the probes to which the target substance has bonded, when the ID information is comprised of the marker the same as or similar to the marker used for marking the target substance.

[0029]

[Description of the Preferred Embodiment]

Now, an analysis method for analyzing organic molecules using a micro-array according to the present invention will be described with reference to the accompanying drawings 1 through 7.

[0030]

First of all, plural types of organic molecules (plural types of cDNAs in this example) are arranged in matrix on a base (or a membrane) using a spotting device. That is to say, plural types of organic molecules 4 are arranged in matrix on the membrane 2 to form a test piece 1 as shown in Figure 1. Each dot on the membrane 2, at which one of the plural types of cDNAs is alloted, can be regarded as a probe.

[0031]

Next, ID information peculiar to the test piece 1 is attached to a predetermined location 20 (see Figure 2) on the test piece 1. Specifically in the present embodiment, a certain radioactive isotope (e.g., ^{14}C, ^{32}P or ^{33}P) is disposed on the predetermined location 20 on the surface of the test piece 1 using the same spotting device as the one used for arranging the probes on the membrane 2. If there was a long time interval scheduled between preparation of the test piece 1 and hybridization thereof, it would be preferable to use ^{14}C which has a long half life. The radioactive isotope used herein that

comprise the ID information to the test piece 1 may be the same radioactive isotope as the one used for marking a target substance on a later stage, or may be a radioactive isotope which is different from the one used for marking the target substance but which emits radiation similar to the radiation emitted by the radioactive isotope used for marking the target substance. Figure 3(a) and Figure 3(b) show exemplary forms of the ID information, i.e., exemplary patterns of the radioactive isotope to be disposed on the predetermined location 20 on the surface of the test piece 1. As shown in Figure 3(a) and Figure 3(b), the ID information peculiar to the test piece 1 is encoded and then attached to the surface of the test piece 1. Specifically, the ID information may include information such as the date of preparing the test piece 1, a serial number, the type of the test piece 1, the types of the substances used as the probes arranged and immobilized to the test piece 1 and the positions of the probes. Any appropriate conventional method may be used for encoding the ID information.

[0032]

The encoded ID information may be arranged in a bar-code pattern as shown in Figure 3(a) or in a dotted pattern as shown in Figure 3(b). In Figure 3(a) and Figure 3(b), the pattern of the ID information is constituted of three portions each representing the date of preparing the test piece 1, the type

of the test piece 1, or the types of the substances used as the probes, respectively. However, the entire contents represented by those three portions may instead be represented by a single portion. The encoding of the ID information peculiar to the test piece 1 enables the test piece 1 to accommodate the ID information in a small area thereon.

[0033]

After arranging the probes on the surface of the membrane 2 following the process explained above, preparation of the test piece 1 is completed by projecting ultraviolet radiation onto the membrane 2 to thereby immobilize the probes to the membrane 2.

[0034]

In the next step, a cDNA marked with a radioactive isotope is synthesized through reverse transcription using as the template poly(A)RNA prepared from the RNA extracted from the cells to be analyzed. This marked cDNA is the target substance in the present embodiment.

[0035]

If there were known information concerning the target substance, it would be desirable to add such information after encoding thereof to the ID information attached to the test piece 1.

[0036]

The test piece 1 is then soaked in a prepared solution, whereby the probes of certain types hybridize with the target substance (see Figure 4). Subsequently, the targets 6 which have not been hybridized are washed away from the surface of the test piece 1 leaving on the surface only those targets 8 hybridized with the probes (see Figure 5). The hybridized targets 8 have been marked with the radioactive isotope.

[0037]

In the next step, the positions of targets 8 left on the test piece 1 are detected by superposing an stimulable phosphor sheet 30 on the test piece 1 (see Figure 6) and leaving the test piece 1 overlaid with the stimulable phosphor sheet 30 in a dark place to expose the stimulable phosphor sheet 30 to the radiation emitted from the radioactive isotope immobilized on the test piece 1. Herein, visible light is projected in advance over the entire surface of the stimulable phosphor sheet 30 to erase unnecessary information stored thereon before superposing the stimulable phosphor sheet 30 on the test piece 1. Accumulated on the stimulable phosphor sheet 30 after a predetermined period of exposure are the radiation energy emitted from the targets 8 left on the test piece 1 and the radiation energy emitted from the radioactive isotope disposed at the location 20 (see Figure 5) as the ID information peculiar to the test piece 1.

[0038]

The term "stimulable phosphor sheet" refers to a phosphor sheet which absorbs and accumulates radiation energy when exposed to radiation and which emits the accumulated radiation energy as light in the manner of stimulated emission when subsequently irradiated with stimulating light such as a laser beam having a certain wavelength range. A typical example of a well-known stimulable phosphor sheet, which is known also as a radiation inverting panel, utilizes a phosphor which exhibits stimulated emission, and is constituted of a base sheet overlaid with a layer of a material composed of a binder and stimulable phosphor particles such as BaFX particles (X represents a halogen atom) dispersed in the binder at a high density.

[0039]

In the next step, the stimulable phosphor sheet 30 is detached from the test piece 1 to measure the radiation energy accumulated thereon. In Figure 7, which illustrates the way of measuring the radiation energy accumulated on the stimulable phosphor sheet 30, the entire surface of the stimulable phosphor sheet 30 is scanned with a stimulation laser beam 10 for readout reflected by a mirror 12, which is either of a half mirror or a dichroic mirror. A spot on the stimulable phosphor sheet 30 irradiated with the stimulation laser beam 10 for readout emits a beam of stimulated emission 14. The beam of stimulated

emission 14 then passes through the mirror 12 and is detected by a photomultiplier (PMT). Accordingly, the positions of the radioactive isotope on the test piece 1 can be determined, because detected by the PMT is the stimulated emission emitted from those spots on the stimulable phosphor sheet 30 corresponding to the positions of the radioactive isotope on the test piece 1. Those spots on the stimulable phosphor sheet 30 include two kinds of spots, i.e., the spots accumulating the radiation energy emitted from the radioactive isotope used as the marker of the targets 8 hybridized with cDNAs 4 arranged in matrix on the test piece 1 in the process of hybridization and the spot accumulating the radiation energy emitted from the radioactive isotope disposed at the location 20 in the pattern of the information peculiar to the test piece 1 arranged and fixed on the test piece 1. The stimulated emission detected by the PMT is then converted to an electric signal. The electric signal is sent to a computer C and the information concerning the emitting positions on the test piece 1 is stored in the computer C.

[0040]

The information concerning the emitting positions stored in the computer C contains the ID information, which is peculiar to the test piece 1 and is attached to the predetermined location 20 in Figure 5, and the information peculiar to the test piece

1 (i.e., the date of preparing the test piece 1, the type of the membrane, the types of the substances used as the probes, the positions of the probes, etc. in this embodiment) can be read out from the ID information.

[0041]

As the first step of analyzing the information stored in the computer C in the present embodiment, the information concerning the emitting positions disposed in a pattern on the location 20 in Figure 5 is analyzed to read out the encoded information indicated by the pattern. Thereafter, the date of preparing the test piece 1, the type of the test piece and the types of the substances used as the probes are identified from the encoded information. After such information is identified, an operator may now recognize which cDNA has or has not hybridized with the RNA extracted from the cells to be analyzed, by inputting to the computer C the information concerning the positions and the types of cDNAs arranged and immobilized on the test piece 1, and having the computer C compare this information with the information concerning the emitting position stored in the computer C.

[0042]

In the analysis system according to the above embodiment of the present invention, in summary, the stimulable phosphor sheet 30 is capable of storing the ID information peculiar to

the test piece 1, i.e., the management information peculiar to the test piece 1 correlated with the ID information (e.g., the date of preparing the test piece 1, the type of the test piece and the types of the substances used as the probes), as the ID information has been attached to the test piece 1 using the radioactive isotope the same as the one used as the marker. Accordingly, the ID information peculiar to the test piece 1 stored on the stimulable phosphor sheet 30 may be detected concurrently with the information concerning the positions of the hybridized probes stored on the same stimulable phosphor sheet.

[0043]

In addition, incorrect association between the ID information peculiar to the test piece 1 and the information concerning the detected positions of the hybridized probes would be prevented effectively, even if there were many types of probes arranged on the test piece 1 and thus there were many types of test pieces to be examined as the test piece 1, as correct association would be achieved referring to the ID information attached to the test piece 1 and obtaining the information concerning the detected positions of the hybridized probes by correlation with the ID information.

[0044]

Moreover, in the above embodiment, there is no need to have

a special reading device for reading out the ID information as the radioactive isotope the same as or similar to the radioactive isotope used as the marker is used for attaching the ID information to the test piece 1, and at the same time, the ID information peculiar to the test piece 1 and the information concerning the positions of the marker of the hybridized probes can be detected simultaneously requiring no additional step.

[0045]

In addition, an ink jet printer may be used in place of the spotting device used in the above embodiment for attaching the radioactive isotope to the membrane 2. In that case, the ID information is printed (attached) on the surface of the membrane 2 using the radioactive isotope as ink.

[0046]

Although the ID information peculiar to the test piece 1 was attached to the test piece 1 before hybridization in the above embodiment, the ID information may instead be attached after hybridization. In that case, the radioactive isotope disposed on the test piece 1 does not require the immobilization process. This is because there is no possibility of the radioactive isotope on the test piece 1 being peeled off after hybridization, while the radioactive isotope may peel off during hybridization or the subsequent process of washing away

the target substance which has not hybridized.

[0047]

In addition, fluorescent dye (e.g., Cy5 or Cy3) may be used in place of the radioactive isotope used in the above embodiment as the marker to mark the target substance. In that case, the PMT can detect the direct information from the test piece without the use of the stimulable phosphor sheet by projecting stimulating light capable of stimulating the fluorescent dye directly onto the test piece 1.

[0048]

Although the radioactive isotope the same as or similar to the radioactive isotope used as the marker is used for attaching the information peculiar to the test piece 1 in the above embodiment, the information peculiar to the test piece 1 may instead be attached through normal printing or a similar method. In addition, the information peculiar to the test piece 1 may be attached by encoding through the method of forming an embossed pattern or an engraved pattern on the membrane 2 or forming a hole.

[Brief Description of the Drawings]

Figure 1 is a perspective view of one embodiment of a test piece according to the present invention,

Figure 2 is a perspective view of one embodiment of the

test piece according to the present invention, schematically showing how organic molecules are arranged and immobilized in matrix on the surface thereof,

Figure 3(a) and Figure 3(b) show exemplary patterns of the encoded information arranged and fixed to the test piece,

Figure 4 is a perspective view of the test piece of Figure 2, schematically illustrating the process of hybridization,

Figure 5 is a perspective view of the test piece of Figure 2 after hybridization,

Figure 6 is a perspective view of the test piece of Figure 2, schematically illustrating the process of exposing an stimulable phosphor sheet superposed on the test piece to the radiation emitted from the radioactive isotope,

Figure 7 is a perspective view of the stimulable phosphor sheet being exposed to a stimulation laser beam for readout, and

Figure 8 is a perspective view of a conventional micro-array base.

[Explanation of the Reference Numerals]

1 test piece

2 membrane

4 cDNAs arranged in matrix

6 target

10 stimulation laser beam for readout

PMT photomultiplier

FIG.1

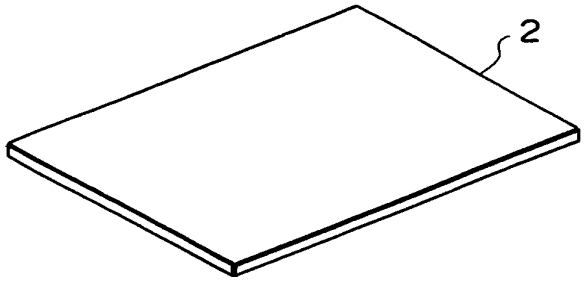


FIG.2

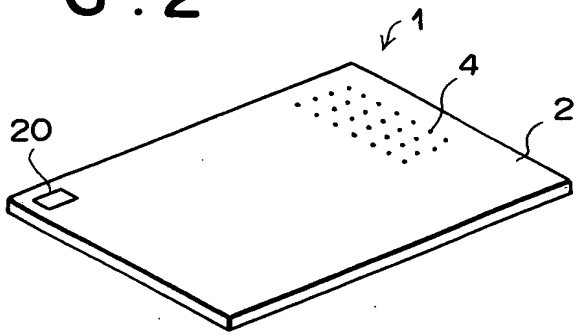
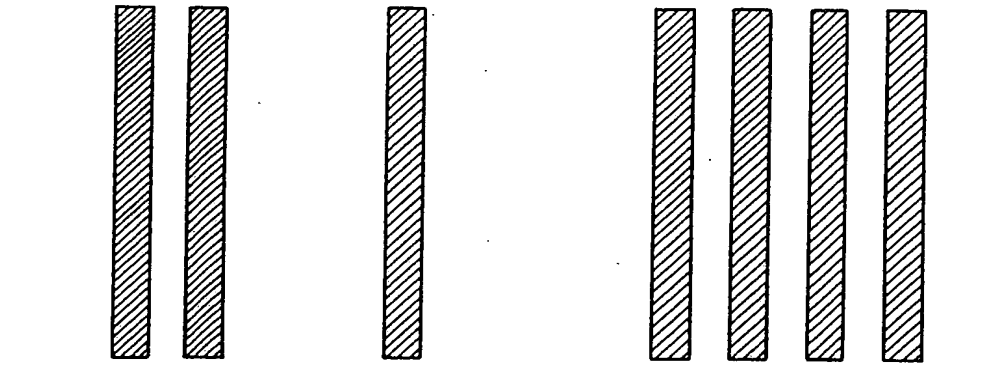
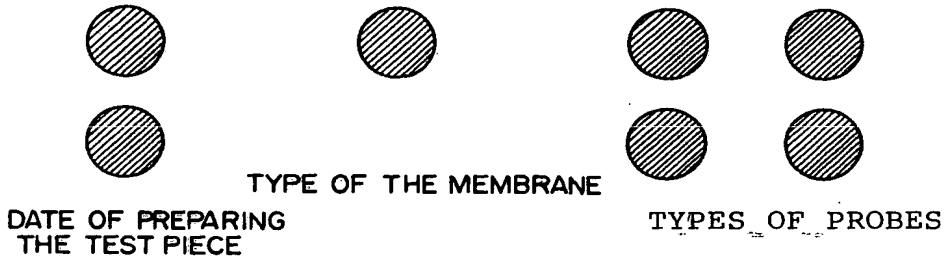


FIG. 3(a)


TYPE OF THE MEMBRANE
TYPES OF PROBES
DATE OF PREPARING THE TEST PIECE

(b)


TYPE OF THE MEMBRANE
TYPES OF PROBES
DATE OF PREPARING THE TEST PIECE

(c)

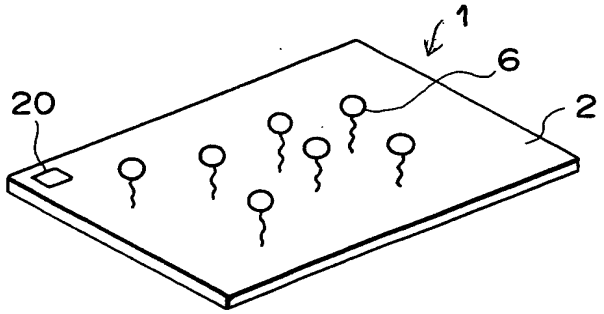

1
6
20
2

FIG. 5

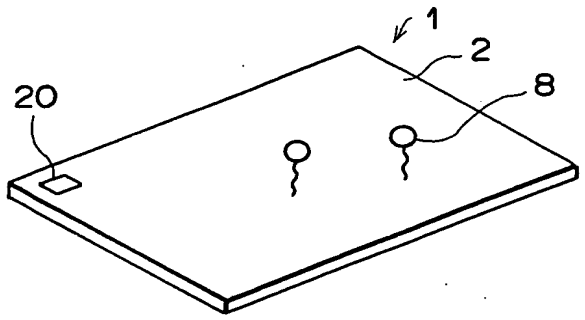


FIG. 6

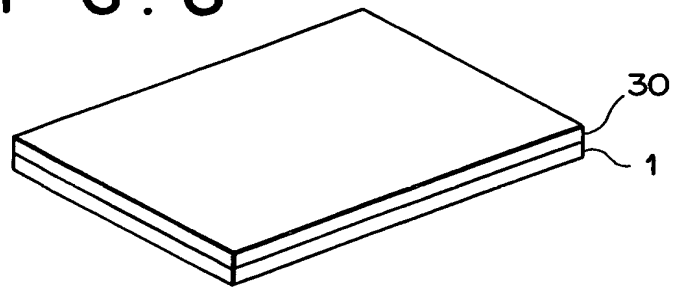


FIG. 7

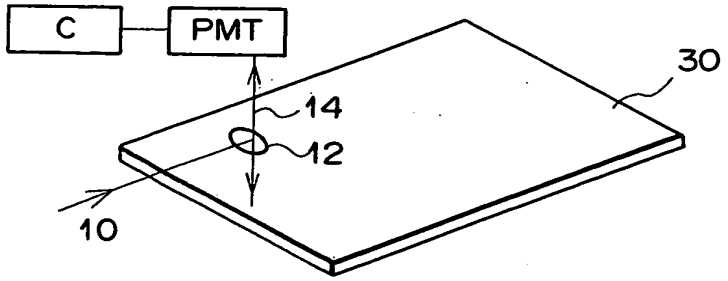
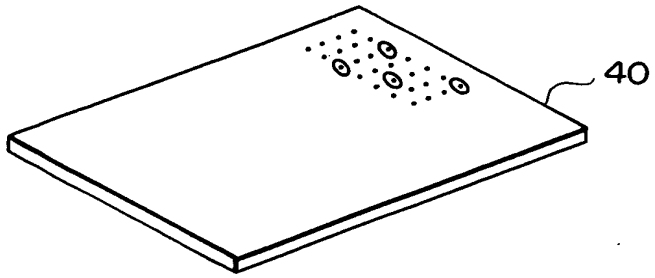


FIG. 8



[Name of Document] Specification

[Abstract]

[Objective]

To prevent incorrect association between the information peculiar to a test piece and the information concerning positions of the probes to which the target substance has bonded in gene expression analysis using a test piece in which probes are arranged in array pattern.

[Constitution]

ID information peculiar to a test piece 1 is attached to the predetermined location 20 on the test piece 1 where plural types of probes 4 to hybridize with a target substance marked with a marker is arranged and immobilized, by using the marker.

[Selected Figure] Figure 2